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Prevalence study of brucellosis among high-risk people in xinjiang region, China

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Abstract

Background: Brucellosis was a zoonotic disease causing serious public health and economic problems.

Methods: Total 2,648 samples were collected between July 2011 and August 2012 from high-risk individuals, who were outpatients at Infectious Disease Hospital of Xinjiang Uygur Autonomous Region. The diagnosis was based upon clinical symptoms and serological or bacteriological test.

Results: 1080 individuals (40.8%) were diagnosed as having been infected with brucellosis. The positive rate of brucellosis diagnosis was correlated with gender, ethnic group and clinical symptoms. All isolates from blood culture were identified as Brucella melitensis biotype 3.

Conclusions: High prevalence of brucellosis exists in Xinjiang, China. An improved healthcare system and preventive measures is needed to minimize the infection of brucellosis.

Keywords: Brucellosis, high-risk groups, prevalence, serological test, bacteriological test

Introduction

Brucellosis was a zoonotic infection caused by *Brucella* resulting in reproductive failure in animals and febrile diseases in humans [1]. Based on sole phenotypic characterization using a range of bacteriological and biochemical tests, *Brucella* was generally classified into six species including *Brucella melitensis, Brucella abortus, Brucella suis, Brucella canis, Brucella ovis* and *Brucella neotomae*, respectively [2]. Over recent years, the number of species has increased to 10 as several new *Brucella* strains had been isolated from marine mammals, rodents, and infected human breast implant, respectively [3,4,5].

An important characteristic of *Brucella* was that they could penetrate the endothelial reticulum and infect macrophages, acting as facultative intracellular parasites [6,7]. Human being can be infected with Brucellosis through various routes eg consumption of contaminated dairy products, microbial inoculation through cuts or abrasions in the skin surface, the conjunctiva inoculation, inhalation of infectious aerosols, accidental human contact with infected animals and consumption of contaminated meat [8]. The common clinical symptoms included weakness, lethargy, chill, fever, sweating, decreased appetite, arthralgia, myalgia, weight loss, headache, back pain and psychological symptoms [6].

The most important first step for control of the disease was accurate diagnosis of brucellosis [9]. Bacteriological detection of brucellosis in humans was the confirming method and depended on the isolation of the bacteria from blood and bacteriological tests and its biotype, which would take at least one week [10]. Due to the adequate sensitivity, simplicity and lower cost, serological tests such as standard agglutination test (SAT), rose bengal test (RBT) and enzyme-linked immunosorbent assay (ELISA). were commonly used for brucellosis screening in laboratory [11].

Xinjiang, the north-western part of China was one of the areas with high prevalence of brucellosis, Many ethnic groups are intermingled and most people are engaged in agriculture and animal husbandry. Traditional habits, limited veterinary support services and husbandry practices aggravated the spread of this disease. In this study, the incidence of brucellosis in outpatients from Xinjiang region and its correlation with gender, ethnic group and clinical symptoms has been investigated. Moreover, the diagnosis value of serological and bacteriological detection on brucellosis has also been evaluated.

Materials and methods

Patients and Samples

All samples of high-risk individuals (n=2,648) were mainly collected between July 2011 and August 2012 from outpatients at Infectious Disease Hospital of Xinjiang Uygur Autonomous Region, Urumqi, China who were also residents in Xinjiang region. These peoples included the patients (n=1,243) with clinical symptoms suggestive of brucellosis such as fever, sweating, arthralgia, myalgia and weakness and individuals (n=1,405) with intimate contact history with individuals who were infected with brucellosis or their family members are infected with brucellosis. The blood samples were taken by the

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Table 1. Brucellosis distribution on Demographic characteristics and common clinical symptoms.

Parameters	Cases(n)	Individuals with non-brucellosis (%)	Individuals with brucellosis (%)	X^2	P
Age					
0-20	350	232(8.8%)	118(4.5%)	27.87	<0.001
21-40	1029	645(24.4%)	384(14.5%)	-	-
41-60	972	530(20.0%)	442(16.7%)	-	-
61 or older	297	201(7.6%)	96(3.6%)	-	-
Gender					
Female	990	707(26.7%)	283(10.7%)	75.75	<0.001
Male	1658	901(34.0%)	757(28.6%)	-	-
Ethnic groups					
Han	1612	1021(38.6%)	591(22.3%)	11.79	<0.01
Ugyur	1036	587(22.2%)	449(17.0%)	-	-
Common clinical symptoms of brucellosis					
Yes	1243	520(19.6%)	723(27.3%)	350.53	<0.001
No	1405	1088(41.1%)	317(12.0%)	-	-

qualified nurses or laboratory technicians. Sera were separated and tested within two hours. The brucellosis was diagnosed according to the criterion published by Ministry of Health of the People's Republic of China.

Rose bengal test (RBT)

The antigen used in the RBT was obtained from the Infectious Disease Institute of Center for Disease Control and Prevention (CDC), Beijing, China. It was prepared and standardized according to the instruction of the manufacturer. Serum samples and antigens were removed from the refrigerator and placed at room temperature for one hour. The test was done by dispensing 0.03 ml of each serum to be tested to an enamel plate. The same amount of Rose Bengal antigen was added to each serum and mixed and shaked by hand for 4 minutes, and the test result was then read. Agglutination appeared as weak positive, positive, strong positive, or very strong positive.

Brucella standard agglutination test (SAT)

The SAT was carried out by double- dilution of serum from 1:25 to 1:800 according to previous report [12]. *Brucella* antigen and control sera were used according to the instruction of the manufacturer. Positive reactions were determined using agglutinoscope, and the titre given indicated the highest dilution in which 50% or more agglutination occurred in the tube.

Culture and identification of organism

A minimum of 8 ml of blood (for children 1-3 ml) was taken through vein puncture and was injected into the culture vials (Peds plusTM/F for children and Plus Aerobic/F for adults, BD BACTECTM, BD company, USA). And then, the culture vials were loaded to BD BACTECTM/FX Culture System(BD company, USA) and monitored for 7 days before vials with negative results were removed according to the procedures outlined by the Clinical and Laboratory Standards Institute. If a vial shows positive result, a 100-μl of blood sample was streaked onto sheep-blood agar plates or *Brucella* medium plate (Mast Diagnostics, UK). Plates were incubated at 37°C aerobically and in the presence of 5-10% of CO2. Plates were examined every 3 days for growth. Typical and well-isolated Brucella-like colony is small, transparent, raised, and convex, with an entire edge and smooth and glistening surface along the streak lines by examining macroscopically by Gram's stain. In addition, bacterial suspension was also prepared and adjusted to 0.5 McBurney unit (about 10^8 cfu/ml) with 4.5% saline for VITEK 2 Gram-Negative Identification Card (VITEK 2 GN Test Kit). The microorganism was identified by the VITEK 2 Identification System (bioMerieux, Inc., France) according to biochemical reactions in the card. The result was reported by the software automatically.

Statistical analysis

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) for Windows, version 12.0. The significance of differences between groups was determined using Chi-square. A value of $P \leq 0.05$ was considered as statistically significant.

Results

Demographic findings

A total of 2,648 individuals of outpatients distributed in seven areas/cities of Xinjiang region, including Urumqi, Yining, Aksu, Hotan, Kaxka, Koria and Turpan were registered. 1080 individuals (40.8%) were diagnosed as infected with brucellosis with ages ranging from 0 to 89 years old. In the 40-61 years-old group, the infection rate of brucellosis reached 16.7% (442 individuals) and significant difference was found between various age groups (X^2=27.87, P<0.001). In all suspected cases, the incidence of brucellosis in male, Uygur people and individuals with clinical symptoms was statistically higher than that in female (X^2=75.75, P<0.001), people of Han nationality (X^2=11.79, P<0.01) and individuals without clinical symptoms (X^2=350.53, P<0.001), respectively (**Table 1**).

Multivariate logistic regression analysis of risk factors

Multivariate logistic regression analysis suggested that gender [odds ratio (OR) 2.09, 95% CI 1.745–2.503, P=0.000)], ethnic groups (OR 1.254, 95% CI1.055-1.492,P=0.01), and clinical symptoms (OR 4.694, 95% CI 3.956–5.569, P=0.000) were highly associated with positive infection of brucellosis. The results were shown in (**Table 2**).

Serological tests

The overall distribution of SAT and RBT results found in the surveyed individuals and the percentages of individuals with a SAT or RBT were shown in (**Table 3**). The percentage of positive titres in SAT (≥50) and RBT were 40.8 % and 40.7%, respectively. There was no statistical difference between SAT and RBT (P>0.05). However, the positive rate of SAT

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Table 2. Multivariate logistic regression analysis.

Variable	S.E	Wald	P	OR	95.0% CI for OR
Gender	092	64.161	.000	2.090	1.745-2.503
Ethnic groups	.088	6.567	.010	1.254	1.055-1.492
Clinical symptoms	.087	314.293	.000	4.694	3.956-5.569

S.E, standard error; OR, odds ratio; 95% CI, 95% confidence interval.

Table 3. Prevalence of SAT, BRT and Microbial culture and identification in individuals with and without clinical symptoms of brucellosis.

Test	Result	Cases(n)	Individuals with clinical symptoms of brucellosis (%)	Individuals without clinical symptoms of brucellosis (%)
SAT	Negative	1568	240(9.1%)	1328(50.2%)
	1:50	225	192(7.3%)	33(1.3%)
	1:100	180	145(5.5%)	35(1.3%)
	1:200	214	162(5.5%)	52(2.0%)
	1:400	396	305(11.5%)	91(3.4%)
	1:>400	65	48(1.8%)	17(0.64%)
	Total positive	1080	852(32.2%)	228(8.6%)
RBT	Negative	1570	289(10.9%)	1281(48.4%)
	Positive	1078	778(29.4%)	300(17.8%)
Microbial culture and identification	Negative	208	35(5.6%)	173(27.7%)
	Positive	416	364(58.3%)	52(8.3%)

was significantly higher than that of RBT in the group of the individuals without clinical symptoms (X^2=12.90, P<0.01).

Brucella bacteriological detection

Blood samples of 624 cases were subject to bacteriological examination. The positive samples of blood culture were inoculated onto the plates of sheep-blood agar and Brucella agar selective media. After 3-4 days of inoculation, the colonies with pinpoint, glistening, smooth and translucent appearance were subject to Gram's stain and only gram-negative coccobacilli were sent to further confirmatory tests. The result demonstrated that the total positive rate of blood culture was 66.7% (416/624) (**Table 3**). The individuals with/without clinical symptoms of brucellosis were 364 (58.3%) and 52 (8.3%) from serological test positive samples, respectively (**Table 3**). The positive percentage of individuals with clinical symptoms was significantly more than that of individuals without clinical symptoms (X^2=297.3, P<0.001). What is isolated from blood was identified as *Brucella melitensis* biotype 3 by the VITEK 2 Identification System.

Discussion

Brucellosis continues to be a major public and animal health problem in many regions of the world. According to World Health Organization (WHO), every year 500,000 people were infected [**13**]. In the USA, 4 to 10% of cases were diagnosed and reported [**14**,**15**]. A total of 189,226 cases of human brucellosis were reported officially, about 90,000 of these were registered between 2000 and 2005 (approximately 15,000 cases per year) in Turkey [**16**]. The prevalence rate of brucellosis in different provinces of Iran varies from 1.5 up to 107.5 per 100,000 persons in 2003 [**17**]. However, the number of reported patients was only 1/25 to 1/10 of the actual number patients of this disease in the community. There was very little information about the prevalence of brucellosis in China.

It was well known that Xinjiang was located at the middle point of the Silk Road and used to be the traffic route linking Rome to Xi'an, China from ante-Christum, surrounded by Mongolia in the east, Russia in the north, Kazakhstan, Kyrgystan and Tadzhikistan in the west and Pakistan, India, Tibet in the south. In the area, Uygur people were dominant (46.4% of all population in Xinjiang) and they are mostly engaged in agriculture and animal husbandry, and the direct contact with animals and animal products was common. Taking unpasteurized dairy products was usual. Furthermore, keeping, slaughtering, and taking of dairy and non-dairy products from livestock were carried out traditionally. The main livestock of the region were sheep, goat, and cattle.

Brucellosis was a zoonosis and, with few exceptions, infection in humans resulted from direct or indirect contact with animal sources. The main source of infection for the general population was dairy products prepared from milk from infected livestock. The milk of infected sheep, goats or cattle may contain large numbers of viable organisms, which become concentrated in products such as yogurt, milk tea, cheese, and etc [**18**]. For good-taste of food, the meat was usually barbecued and half-baked. In this study, the incidence of brucellosis in Uygur people was higher than that in Han people (P<0.01), and highest in patients aged between 40 and 60 years (16.7%). It may attribute to different habits between both ethnic groups.

In addition, direct contact with livestock was a well documented source of infection. Infection might occur through cuts and abrasions on the skin, via the conjunctiva, and by inhalation. These routes of infection were quite common for farmers, veterinarians, and butchers, who were all exposed to higher risk of infection through their contact with animals and animal products. In the surveyed outpatients, most of them were engaged in high-risk occupations as described above and the percentage of brucellosis reached 40.8%. The positive rates of male and individuals with clinical symptoms were significantly higher than that of female and those without clinical symptoms (P<0.001, P<0.001, respectively). The result was correlative with Uygur tradition that men dominantly worked outside and infection risks increased. Multivariate logistic regression analysis demonstrated that gender, ethnic group, and clinical symptoms were highly associated with positive rate of brucellosis (**Table 2**).

The brucellosis in human has a diverse range of clinical symptoms, and the most important of them was undulant fever and arthrodynia [**19**,**20**]. Substantial proportion

of patients were accompanied with splenomegaly and/or hepatomegaly. When the disease became chronic, it would affect almost all organs and resulting in a complicated syndrome including spondylitis, endocarditis, and meningoencephalitis [19]. Antibiotics regimens eg Doxycycline 1 g twice a day and rifampicin 600 mg daily for 3-6 monthes was usually used in the treatment of acute patients with brucellosis.

The diagnosis of human brucellosis was not difficult if the level of suspicion was high and the symptom was typical, but the varied manifestations for localized, sub-acute, or chronic infection made it prone to be misdiagnosed [21-23]. As the symptoms of brucellosis were non-specific, the diagnosis could be done based upon laboratory finding only [24].

Serological test such as SAT and RBT were useful methods for indirect diagnosis or screening of brucellosis. The positive percentage of SAT and RBT in this study were 40.8 % and 40.7%, respectively (**Table 1**). No significant difference was found between two methods (P>0.05). However, the *Brucella* antibodies detected in asymptomatic individuals could be related to a history of exposure, inactive brucellosis, or repeated exposure to antigenic stimuli [25]. The positive percentage of SAT and RBT in individuals without clinical symptoms were 21.1 % and 27.8 % of positive cases, respectively (**Table 3**). It might reflect that antibody profiles did not have specific clinical correlations, and titers often remained high for a protracted period. Generally, IgM against *Brucella* would appeare as early as the first week after being infected, followed by an IgG amplification at the second week. Both classes of immunoglobulin peaked during the fourth week and the antibiotics application was accompanied with a decline in both IgM and IgG titers. IgM titers persisted at levels that were higher than those of IgG titers for more than six months, and both classes were persistent for almost one year [25].

The seropositivity of SAT in individuals with clinical symptoms was statistically higher than that of RBT (P<0.01). It may be attributed to the fact that the higher titer of antibody in the individuals with clinical symptoms was double-diluted to proper concentration to antigen in SAT, and avoiding of "hook effect" resulting in immunological reaction which was easy to be observed.

Bacteriological examination was regarded as the "golden standard" method in brucellosis diagnosis, although the bacteriological isolation and identification was a time-consuming procedure. The initial isolation of bacteria was a big problem in diagnostic laboratories [26]. Fortunately, the professional blood-culture vials and culture system had been applied generally in clinical laboratories, the positive percentage of blood culture has dramatically increased in current years. In the study, the majority of samples subject to blood culture was from patients with high suspicion of brucellosis, who had an epidemiologic history, severer clinical symptoms and/or high titer of antibody in serological test. Total positive rate of bacteriological culture was 66.7% (416/624), reaching 58.3% in samples of individuals with clinical symptoms, which was significantly higher than that of individuals without clinical symptoms (P<0.001).

Brucella melitensis, Brucella abortus, and Brucella suis were the three species associated with human disease. In this study, What was isolated were identified as *Brucella melitensis* biotype 3, which implied sheep or goat as potential source of infection for brucellosis in Xinjiang area of China.

In conclusion, although brucellosis had been, or was close to being eradicated from a number of developed countries, it continued to be a major public and animal health problem in many regions in the developing countries of the world. In Xinjiang area, human cases continued to occur due to their traditional use of raw milk products or eating the half-baked meat and having close contact with infected animals or people. A well developed healthcare system and preventive measures would help to reduce potential infection risks and decrease incidence of brucellosis.

Competing interests
The authors declare that they have no competing interests.

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